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Impact of Ion-Pair Reagents on LC/MS Analysis

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Introduction

Ion-pair chromatography (IPC) is typically done when the analytes of interest include basic or ionic compounds that are difficult to chromatograph by reverse-phase LC. In IPC, the mobile phase contains an ion-pair reagent that attaches to the stationary phase and creates a charged surface. The sample ion exchanges with the counter ion of the ion-pair reagent thus increasing interaction with the column resulting in greater retention of the sample.[1] Ion-pair reagent type and concentration, as well as mobile phase pH, affect IPC. The most commonly used ion-pair reagents are either alkyl sulfonates or tetraalkyl ammonium salts, which are typically nonvolatile.

With the increasing use of LC/MS, it is important to consider the impact of ion-pair reagent on both the MS hardware and signal. Atmospheric pressure ionization (API) LC/MS techniques require that the analyte be an ion in solution (electrospray) or that the analyte accepts or donates a proton in the gas phase (atmospheric pressure chemical ionization). Ion pairing can interfere with this ionization process, thus affecting the LC/MS analysis. This work examines the impact of ion-pair reagent selection on LC/MS analysis using a variety of analytes.

Materials and Methods

All experiments were done on an Agilent 1100 Series LC/MSD system that was comprised of a binary pump, vacuum degasser, autosampler, thermostatted column compartment with column-switching valve, diode-array detector, and LC/MSD. The LC/MSD was used with either the electrospray ionization (ESI) or atmospheric pressure chemical ionization (APCI) source. Complete system control and data evaluation was done on the Agilent ChemStation for LC/MS.

Reagent grade chemicals and HPLC grade solvents were used for preparing mobile phases. Buffer and ion-pair reagent were added to both aqueous and organic mobile phases to ensure a constant concentration during gradient chromatography. A high concentration (10 mM) of ion-pair reagent was used to challenge the LC/MS system. For negative mode experiments, 10 mM acetic acid was also added to control the pH of the mobile phase. The same gradient was used for all mobile phases so that the extent of ion pairing could be verified.

A Zorbax® Rapid Resolution XDB-C8 cartridge column (2.1 × 30 mm) was used for all experiments. A new column was used for each of the different ion-pair containing mobile phases to avoid mixed behavior.

For positive mode, weak and strong volatile ion-pair reagents (valeric acid and perfluoroheptanoic acid respectively) were compared to a classic nonvolatile strong ion pair reagent (sodium heptane sulfonic acid). In negative mode, the strong, volatile ion-pair reagent tributylamine was compared to tetrabutylammonium hydroxide, a classic nonvolatile, strong ion-pair reagent.

Analytes were selected that would show different strengths of interaction with the ion-pair reagents. For positive ion mode (Figure 1), both APCI and ESI-LC/MS analyses were performed for all conditions. In negative ion mode, only two of the analytes (Figure 2) ionized by APCI so no studies were done in negative mode APCI. Triplicate injections of a 10 ng/µl mixture of the analytes were done for each condition.

In addition to testing the different ion-pair reagents, a long-term stability study (15 hr) was done by positive mode ESI-LC/MS with heptane sulfonic acid using erythromycin (0.2 ng/µl) as the analyte.

Results and Discussion

For each ion-pair reagent tested, average retention time and MS response were calculated for each analyte. These results were used to evaluate the effectiveness of the different ion-pair reagents, the strength of ion-pair interaction with the different analytes, and the impact of the ion-pair reagent on ionization of the analytes.

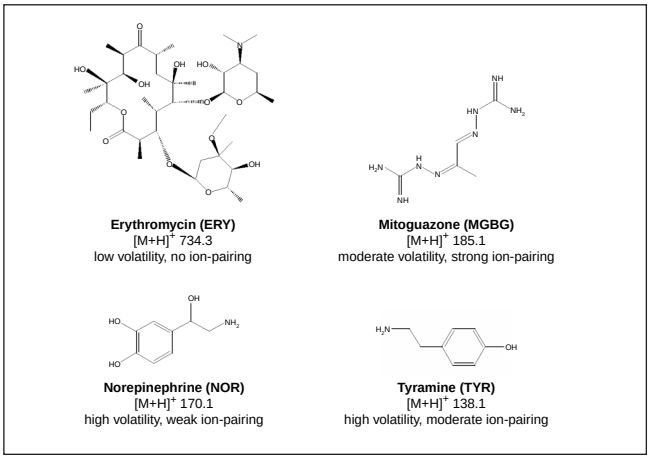

Figure 1. Positive mode analytes.

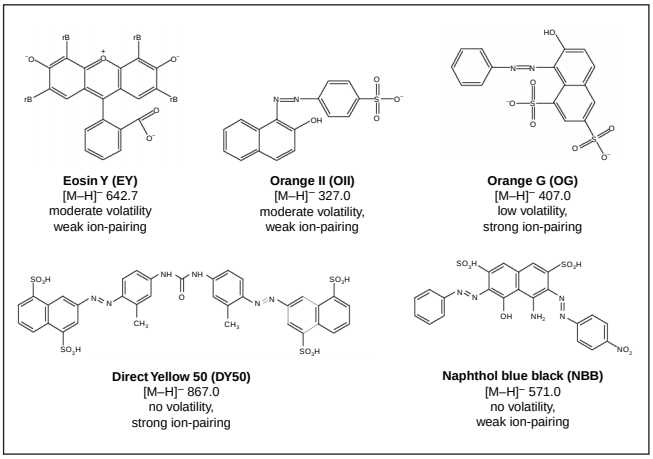

Figure 2. Negative mode analytes.

Effect on Chromatographic Retention

Retention behavior indicates both the strength of the ion-pair reagent and the strength of the interaction with different types of analytes. The retention behavior with and without ion-pair reagent was compared by calculating relative retention as follows: $(RT_{ion\text{-}pair} - RT_{AmOAc})/ RT_{AmOAc}$.

In positive mode, erythromycin shows no ion pairing as the relative retention is unchanged under all conditions (Figure 3). Norepinephrine, tyramine and mitoguazone all showed increased retention with the strong ion-pair reagents, perfluoroheptanoic acid (PFHA) and heptane sulfonic acid (HSA). Valeric acid (VA), a weak ion-pair reagent, showed almost no effect on relative retention. In negative ion mode, all of the analytes show some degree of ion pairing (Figure 4). However, orange II, naphthol blue-black, and eosin Y show only slight ion-pairing while direct yellow 50 and orange G strongly ion-pair with both reagents.

For both positive and negative mode, a volatile strong ion-pair reagent was shown to be equivalent to a classic nonvolatile ion-pair reagent.

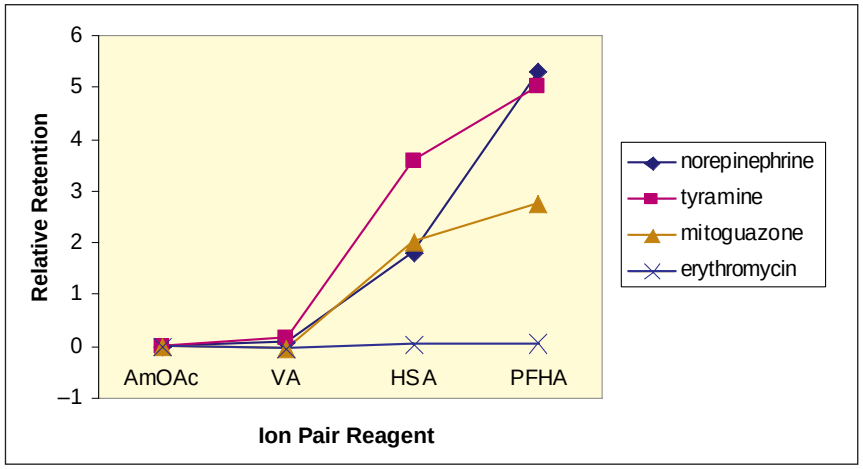

Figure 3. Effect of positive mode ion-pair reagents on relative retention. Ion-pair reagents: valeric acid (VA), heptanesulfonic acid (HSA), and perfluoroheptanoic acid (PFHA).

Chromatographic Conditions

Column:	30 × 2.1 mm Zorbax® Rapid Resolution XDB-C8, 3.5 µm (p/n 873700-906)
Mobile phase:	A = 10 mM each ammonium acetate and ion-pair reagent in water B = 10 mM each ammonium acetate and ion-pair reagent in methanol
Gradient:	start with 0% B at 3 min 100% B
Flow rate:	0.6 ml/min
Column temp:	40°C
Injection vol:	1 µl
Diode-array detector:	signal 254, 20nm; reference 360, 100 nm

ESI MS Conditions

Source:	ESI
Ion mode:	positive
Vcap:	4000 V
Nebulizer:	35 psig
Drying gas flow:	13 l/min
Drying gas temp:	350°C
SIM ions:	138.1, 170.1, 185.1 and 734.3
Stepsize:	0.1
Peakwidth:	0.07 min
Time filter:	on
Fragmentor:	variable — 30 V (138.1, 185.1); 10 V (170.1); 80 V (734.3)

APCI MS Conditions

Source:	APCI
Ion mode:	positive
Vcap:	4000 V
Nebulizer:	60 psig
Drying gas flow:	4 l/min
Drying gas temp:	350°C
Corona:	4 µA
Vaporizer:	350°C
SIM ions:	138.1, 170.1, 185.1 and 734.3
Stepsize:	0.1
Peakwidth:	0.07 min
Time filter:	on
Fragmentor:	variable — 30 V (138.1, 185.1); 10 V (170.1); 80 V (734.3)

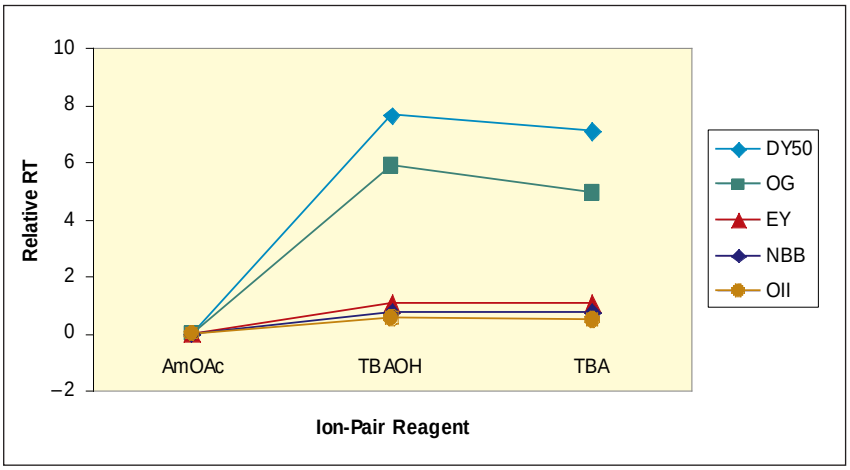

Figure 4. Effect of negative mode ion-pair reagents on relative retention. Ion-pair reagents: tributylamine (TBA) and tetrabutylammonium hydroxide (TBAOH).

Chromatographic Conditions

Column:	30 × 2.1 mm Zorbax® Rapid Resolution XDB-C8, 3.5 µm (p/n 873700-906)
Mobile phase:	A = 10 mM each ammonium acetate, acetic acid and ion-pair reagent in 40% methanol in water except for control, which was 20 mM ammonium acetate only
	B = 10 mM each ammonium acetate, acetic acid and ion-pair reagent in methanol except for control, which was 20 mM ammonium acetate only
Gradient:	start with 0% B at 3 min 100% B
Flow rate:	0.6 ml/min
Column temp:	40°C
Injection vol:	1 µl
Diode-array detector:	signal A: 280, 20 nm; signal B: 484, 20 nm; signal C: 517, 20 nm; signal D: 618, 20 nm; signal E: 390, 20 nm

ESI MS Conditions

Source:	ESI
Ion mode:	negative
Vcap:	4000 V
Nebulizer:	35 psig
Drying gas flow:	13 l/min
Drying gas temp:	300°C
SIM ions:	327.0, 406.9, 517.0, 646.6 and 867.0
Stepsize:	0.1
Peakwidth:	0.1 min
Time filter:	on
Fragmentor:	variable — 70 V (406.9); 90 V (327.0, 571.0, 646.6); 160 V (867.0)

Effect on MS Signal

The impact of the ion-pair reagent on MS response was calculated as a percent of the control (ammonium acetate only) for each analyte using the average of the triplicate injections. In positive mode, the APCI signal for norepinephrine, the most volatile analyte, increases in the presence of both volatile ion-pair reagents (VA and PFHA) because the reagents assist in the ionization process by serving as strong gas-phase acids (Figure 5). Nonvolatile HSA does not improve the norepinephrine signal. The APCI response for erythromycin, which has low volatility, is greatly reduced by HSA (nonvolatile) and PFHA (low volatility) because these ion-pair reagents trap the analyte in the dried droplets. MGBG, which strongly ion-pairs, shows a greatly reduced response in the presence of both HSA and PFHA. The ESI signal for norepinephrine is improved by the presence of the volatile ion-pair reagents (VA and PFHA). Ionization of erythromycin is also improved when PFHA is present. HSA causes significant suppression for all analytes because the nonvolatile ion-pair hinders the escape of analyte from the droplets during electrospray ionization.

The effect of the ion-pair reagents in negative mode ESI (Figure 6) was calculated as with the positive ion mode experiments described above. The ESI signals for direct yellow 50 and orange G are improved by the presence of tributylamine (TBA), as a result of a pH effect in the drying ESI droplet. As the droplet evaporates, the acetic acid is removed faster than TBA, thus increasing the pH in the droplet and improving ionization. Tetrabutylammonium hydroxide (TBAOH) causes significant suppression for all analytes because the nonvolatile ion-pair hinders the escape of analyte from the droplets during electrospray ionization.

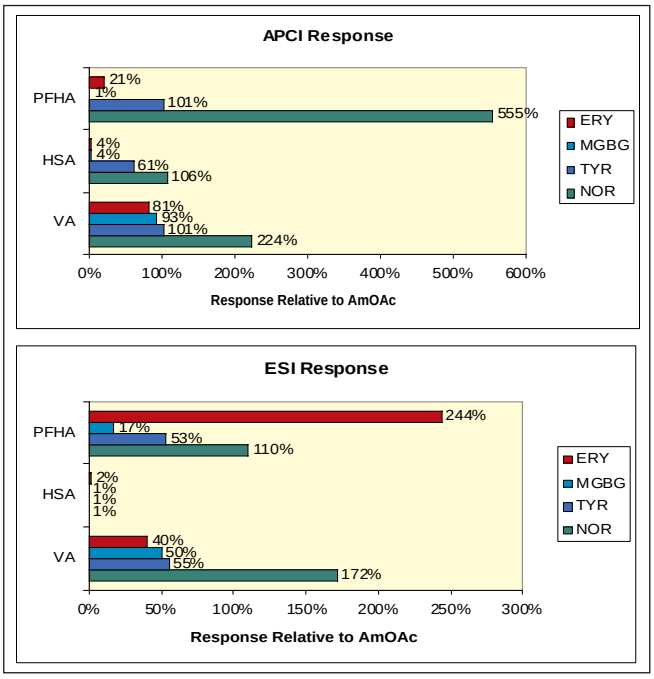

Figure 5. Effect of positive mode ion-pair reagents on MS signal for ESI and APCI.

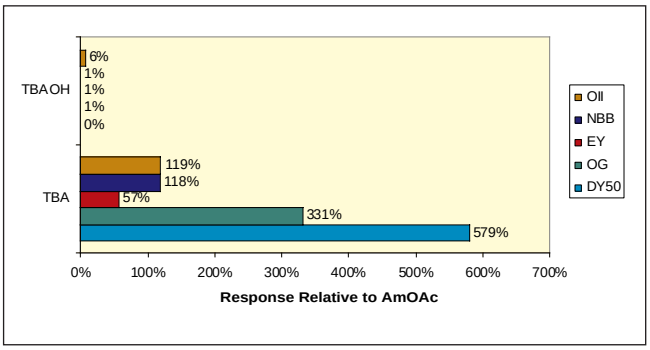

Figure 6. Effect of ion-pair reagents on negative mode MS signal.

Stability Study

While ion pairing can reduce the MS response, it is also sometimes necessary for the chromatographic separation. A long-term stability study was done using HSA and erythromycin to demonstrate the compatibility of the LC/MS system with ion-pairing methods. Because the nonvolatile ion-pair reagent will deposit on the end cap, a flush method was developed to automatically clean the source. The column-switching valve in the thermostatted column compartment was used to bypass the column. The flush method used no column and pumped water at a high flow rate for two minutes. This resulted in a large amount of condensation in the source that served to flush any deposits off the end cap. The method then stopped the LC flow for one minute, which allowed the source to dry. This flush method was automatically run after every 20 injections. The results of this study (Figure 7) show that the LC/MS system is capable of running ion-pairing chromatography overnight.

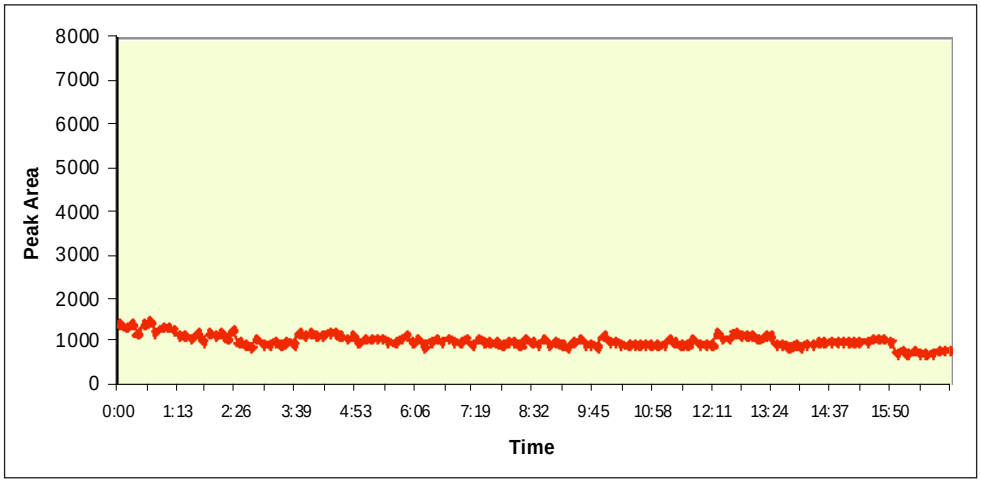

Figure 7. Stability of MS response using 1 ng erythromycin on-column with 10 mM heptane sulfonic acid in the mobile phase.

ANALYSIS METHOD:

Chromatographic Conditions

Column:	30 × 2.1 mm Zorbax® Rapid Resolution XDB-C8, 3.5 µm (p/n 873700-906)
Mobile phase:	A = 10 mM each ammonium acetate and HSA in water B = 10 mM each ammonium acetate and HSA in methanol
Isocratic:	55% B
Flow rate:	0.6 ml/min
Column temp:	40°C
Injection vol:	5 µl
Diode-array detector:	signal 254, 20nm; reference 360, 100 nm

ESI MS Conditions

Source:	ESI
Ion mode:	positive
Vcap:	4000 V
Nebulizer:	35 psig
Drying gas flow:	13 l/min
Drying gas temp:	300°C
SIM ion:	734.3
Stepsize:	0.1
Peakwidth:	0.1 min
Time filter:	on
Fragmentor:	80 V

FLUSH METHOD:

Chromatographic Conditions

Column:	none
Mobile phase:	A = water
Isocratic:	100% A
Flow rate:	initial 3 ml/min, 5 ml/min from 0.5 to 2 min, then 0 ml/min

ESI MS Conditions

Source:	ESI
Ion mode:	positive
Vcap:	0 V
Nebulizer:	35 psig
Drying gas flow:	13 l/min
Drying gas temp:	300 °C

Impact of Ion-Pair Reagents on LC/MS Analysis

Conclusions

The Agilent 1100 LC/MSD system is clearly compatible with LC methods in which ion-pair chromatography is used. The long-term stability of the system was demonstrated using 10 mM nonvolatile ion-pair reagent in the mobile phase, showing that it is feasible to do overnight, routine work using ion-pair chromatography.

The challenge for the analyst is to find an ion-pair system that does not interfere with the ion formation process but still yields acceptable chromatographic behavior. The ion formation process is complicated and involves the analyte, choice of ion-pair reagent, and ionization mode. Which ionization technique (APCI or ESI) works best will depend on the interplay of these factors.

This work also demonstrates that there are volatile ion-pair reagents that can be substituted for more traditional nonvolatile ion-pair reagents. These volatile ion-pair reagents still allow manipulation of retention time while reducing the possibility of suppressing MS ionization.

References

1. Snyder, L. R., Kirkland, J. J., and Glajch, J. L., *Practical HPLC Method Development,* 2nd ed., John Wiley & Sons, Inc., New York, 1997, pp. 317–341.

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